Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1218259

Anatolian Curr Med J 2023; 5(1); 34-38

Comparison of different endometrial preparation protocols in frozen-thawed embryo transfer cycles in women with polycystic ovary syndrome

Murat Önal[1], Mehmet Ağar[2], Ayşe Şeyma Küçükakça[3], Tuğba Gürbüz[4]

[1]Gynolife IVF Clinic, Lefkosa, Cyprus
[2]Private Clinic, Şanlıurfa, Turkey
[3]Department of Gynecology and Obstetric Clinic, Istanbul Medipol University Çamlıca Hospital, İstanbul, Turkey
[4]Gynecology and Obstetric Clinic, Medistate Hospital, İstanbul, Turkey

Cite this article as: Önal M, Ağar M, Küçükakça AŞ, Gürbüz T. Comparison of different endometrial preparation protocols in frozen-thawed embryo transfer cycles in women with polycystic ovary syndrome. Anatolian Curr Med J 2023; 5(1); 34-38.

ABSTRACT

Aim: This study aimed to evaluate the most suitable endometrial preparation protocols such as hormone replacement therapy (HRT) with gonadotropin releasing hormone analogue (GnRH-a) suppression, HRT without GnRH-a suppression and mild ovarian stimulation (OS) for women with polycystic ovary syndrome (PCOS) undergoing frozen-thawed embryo transfer (FET).

Material and Method: We conducted a historical cohort analysis of 161 women with PCOS who underwent the "freeze-all" strategy between December 2018 and August 2020 because of their high risk for ovarian hyperstimulation syndrome. Three endometrial preparation protocols were used: HRT with GnRH-a suppression (n=43); HRT without GnRH-a suppression (n=86); mild-OS (n=32).

Results: The biochemical pregnancy results (55.8 % vs 54.65 % vs 53, p=0.900), ongoing pregnancy rates (44.2 % vs 43 % vs 40.62, p=0.572), and abort rates (20.8 % vs 21.3 % vs 23.52, p=0.900) were similar between the HRT with GnRH-a suppression, without GnRH-a suppression and mild-OS, respectively. This study showed no statistically significant difference between the three protocols in laboratory parameters (p>0.05).

Conclusion: There was no statistically difference between three groups in terms of pregnancy outcomes. Dependent on clinical experience and facility, one of these protocols could be deployed for FET in women with PCOS.

Keywords: Frozen-thawed embryo, GnRH-a, implantation, polycystic ovary syndrome

INTRODUCTION

Frozen embryo transfer (FET) is generally employed in assisted reproductive medicine due to its ability to lower the risk of ovarian hyperstimulation syndrome (OHSS) improving the reproductive outcomes (1). FET as an alternative to fresh cycle transfer has been suggested to be applied for women with polycystic ovary syndrome (PCOS) because the significantly increasing risk of OHSS under this condition (2). In fact, there is much proof for a considerable advantage of this method for women with PCOS (1).

Depending on the diagnostic criteria, PCOS affects 5%-18% of reproductive-aged women worldwide (3). PCOS, as a common disorder, has a relationship with infertility (4,5). It is essential to identify the importance of the factors such as types of endometrial preparation protocols that affecting the success of assisted reproductive methods in women with PCOS.

The ideal endometrial preparation protocol should be considered for women with PCOS. Different strategies for endometrial preparation have been described, including a natural modified cycle (NMC) where hCG is administered to design embryo transfer (ET) rather than measuring luteinizing hormone (LH), a purely natural cycle (NC) with detection of LH in blood or urine, artificial cycle with progesterone (P4), and estradiol (E2), hormone replacement therapy (HRT) with or without gonadotropin-releasing hormone (GnRH) analogs and stimulated cycles with low doses

Corresponding Author: Tuğba Gürbüz, drtgurbuz@hotmail.com

Received: 13.12.2022 **Accepted:** 23.12.2022

This work is licensed under a Creative Commons Attribution 4.0 International License.

of gonadotropins (6,7). In the latest meta-analysis, the use of one strategy over others is not supported, but using the pure NC over the NMC or a NC with progesterone over the NC have been supported by other authors to report better results (8,9). Several approaches in artificial or natural preparation has also been shown in surveys, including 179 centers in the world. One can find several different approaches in answers about preparation of FET and in questions such as if its timing in an artificial or a natural cycle shows various responses and if P4 is needed (6).

The comparison of the method of endometrial preparation in reproductive-aged women has been evaluated in many studies, and different results have been reported (10-13). Infertility is more prevalent in women with PCOS, and they need more assisted reproduction technology (14,15). However, the comparison of these methods in women with PCOS is less studied, and there is a need for serious research in this field.

The prominent importance of endometrial preparation protocols in the favorable pregnancy outcomes is known. However, the best protocol for women with PCOS who experience FET cycles is still uncertain. Women with PCOS do generally not have a regular menstrual cycle, so the NC or NMC protocol will not be the most suitable choice for women with PCOS (16). For this reason, this protocol was not examined in this research. This study aimed to evaluate the suitable endometrial preparation protocols such as HRT with GnRH-a suppression, HRT without GnRH-a suppression, and mild ovarian stimulation (OS) for women with PCOS undergoing FET.

MATERIAL AND METHOD

This study was carried out with the permission of Beykoz University Research and Project Development Ethics Committee (Date: 26.10.2020, Decision No:1). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

One hundred sixty one women participated in this study between December 2018 and August 2020. In this period, 851 patients who underwent FET were examined, and 161 women with PCOS aged between 20 and 35 were included. In 43 patients, it was employed the HRT with GnRH-a suppression (Group:1), in 86 patients, it was employed the HRT without GnRH-a suppression (Group:2), and in 32 patients, it was employed the Mild ovarian stimulation (Group:3).

The exclusion criteria were as follows: 1) known chronic disease, 2) over 35 years of age, 3) body mass index (BMI)>30,4) Having additional infertility factors other than PCOS, and 5) 2 or more failed attempts.

The inclusion criteria were as follows: 1) 20-35 years old, 2) Anti- mullerian hormone (AMH)>5 ng/ml, 3) Women with PCOS according to Rotterdam criteria, 4) Those who have no previous attempts or at most one attempt, and 5) Top-Good Quality (5AA-5AB-4AA-4AB) single blastocyst transfer.

Endometrium Preparation Protocols

The three primary endometrial priming protocols for FET were HRT, mild-OS, and NC. Among these protocols, mild-OS is employed less than the other two (7). HRT cycles can be used with or without GnRH-a for pituitary suppression (17).

Statistical Analysis

The Kolmogorov-Smirnov test performed to check the normality, and the nonparametric tests performed given the non-normality of the groups before the statistical analyses. Mean and standard deviations (SD) measured to check each continuous variable, including age, BMI, total oocytes, MII oocytes, , multi-pronuclei (PN), AMH, prolactin, Free T4 (FT4), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), E2, and endometrial thickness. The Kruskal Wallis-H test deployed to examine the difference between the three endometrial preparation protocols. Chi-square tests were applied to describe the relationship between proportions of categorical variables such as pregnancy results, ongoing pregnancy rate and abort rate. SPSS v24 used for statistical analyses. A value of p-value < 0.05 was accepted as statistically significant.

We utilized the G-Power 3.1 program to calculate the example size. The two groups' total mean was calculated based on the Mann-Whitney test with a power of 90%, an effect size of 50%, and a 0.05 type 1 error for at least 146 patients (18).

RESULTS

This study included One hundred sixty one age-matched (30.75±3.39) and BMI-matched (23.78±2.28) women. 43 patient in the first group with the mean age (30.34±3.90), 86 patient in the second group with the mean age (30.39±3.64), and 32 patient in the third group with the mean age (30.37±4.30) were compared with each other. **Table 1** shown information about the descriptive statistics of maternal characteristics and laboratory parameters. We compared laboratory parameters between three groups and assessed the capability of those parameters to differentiate between groups.

Table 1. Descriptive statistics of study parameters in women with PCOS (n=161)

Study parameters	median (range) mean ± SD
Maternal characteristics	
Age	32(20-35)30.38±3.83
BMI	24(19.8-29)24±2.2
Laboratory parameters	
Total oocytes	0(0-1)0.34±0.48
MII oocytes	5(5-7)5.19±0.44
PN	9(7-16)10.25±2.49
AMH	8(6-15)9.07±2.38
Prolactin	8(6-14)8.51±2.07
FT4	15(8.48-25)16.76±5.51
TSH	1(0.31-1.62)1.04±0.27
FSH	1.21(0.63-2.46)1.49±0.53
LH	7.42(4-12)7.56±1.26
E2	9(3.52-17)8.68±2.54
Endometrial thickness	41(30-51.2)40.05±6.5

SD, standard deviation; AMH, Anti-Mullerian hormone; PN, multi-pronuclei; FT4, Free T4; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, Estradiol.

As stated in **Table 2**, a Kruskal Wallis-H test did not find a statistically significant association between the three treatment groups in regard to age and BMI (p>0.05). AMH of first group (mean = 5.21) were comparable than the second group (mean = 5.17) and the third group (mean = 5.19). A Kruskal Wallis-H test indicated that this difference was not statistically significant (p>0.05). No significant difference was observed between the three groups regarding total oocytes, MII oocytes, PN, prolactin and FT4 (p>0.05). TSH, LH and FSH levels were similar between the three groups (p>0.05). There was no statistically significant difference between groups in terms of E2 and endometrial thickness (p>0.05).

Table 2. Comparison of study parameters between three groups

Study parameters	first group (n=43) mean ± SD	second group (n=86) mean ± SD	third group (n=32) mean ± SD	p
Age	30.35±3.9	30.4±3.65	30.38±4.31	0.776
BMI	24.16±2.07	23.85±2.22	24.16±2.39	0.453
AMH	5.21±0.52	5.17±0.41	5.19±0.4	0.797
Total oocytes	10.23±2.48	10.24±2.41	10.28±2.79	0.792
MII oocytes	9.05±2.5	9.09±2.35	9.03±2.36	0.925
PN	8.51±2.11	8.5±1.97	8.53±2.34	0.934
Prolactin	16.61±5.55	16.85±5.47	16.7±5.72	0.940
FT4	1.05±0.25	1.04±0.28	1.05±0.27	0.970
TSH	1.51±0.53	1.49±0.54	1.46±0.51	0.973
FSH	7.52±0.97	7.55±1.4	7.61±1.26	0.814
LH	8.72±2.65	8.67±2.23	8.67±3.18	0.941
E2	40.02±6	40.03±6.69	40.16±6.83	0.997
Endometrial thickness	9.95±1.05	9.92±1.05	9.94±1.08	0.975

M, Mean; N, number of subjects; AMH, Anti-Mullerian hormone; PN, multi-pronuclei; FT4, Free T4; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, Estradiol; All variables tested by a Mann-Whitney U test.

As stated in **Table 3**, a chi square test found that there was not a statistically significant association between the pregnancy results (biochemical and ongoing) and the three treatment groups (HRT with GnRH-a suppression, HRT without GnRH-a suppression, and mild-OS) (p>0.05).

Table 3. The relationship between pregnancy (biochemical and ongoing) results and the three groups

Variables		First group (n=43) n(%)	Second group (n=86) n(%)	Third group (n=32) n(%)	p
Pregnancy results Bhcg(+)(%)	Yes	24 (55.8)	47 (54.65)	17 (53)	0.900*
	No	19 (44.2)	39 (45.35)	15 (47)	
Ongoing pregnancy rate(%)	Yes	19 (44.2)	37 (43)	13 (40.62)	0.572*
	No	24 (55.8)	49 (57)	19 (59.38)	

*A Chi-square test

As stated in **Table 4**, a chi square test found that there was not a statistically significant association between the abort rate and the three treatment groups (p>0.05).

Table 4. The relationship between abortion rate and the three groups

Variable		First group (n=24) n(%)	Second group (n=47) n(%)	Third group (n=17) n(%)	p
Abortion rate (%)	Yes	5(20.8)	10(21.3)	4(23.52)	0.249*
	No	19(79.2)	37(78.7)	13(76.48)	

*A Chi-square test.

DISCUSSION

The present study investigated whether biochemical pregnancy results, ongoing pregnancy rates, and abort rates varied when three different endometrial preparation protocols were employed for FET in women with PCOS. Therefore, we retrospectively examined our data of FET cycles and included three endometrial preparation protocols in this study: HRT with GnRH-a suppression, without, and mild-OS. Our results indicate that overall, patients with programmed HRT with or without GnRH-a suppression did not have higher biochemical pregnancy results, ongoing pregnancy rates, and abort rates compared with patients with mild-OS.

In our study, biochemical pregnancy results (55.8 % vs 54.65 % vs 53, p=0.900), ongoing pregnancy rates (44.2 % vs 43 % vs 40.62, p=0.572), and abort rates (20.8 % vs 21.3 % vs 23.52, p=0.900) were similar between the HRT with GnRH-a suppression, without GnRH-a suppression and mild-OS, respectively. The ongoing pregnancy rates and abort rates was relatively low in the mild-OS compared to the HRT protocols. This study showed no statistically significant difference between the three protocols in laboratory parameters.

By studying the literature, we found that many studies have been performed comparing women's fertility outcomes of different endometrial preparation protocols. However, studies that have studied women with PCOS are limited. The comparison of endometrial preparation protocols in women with PCOS is a new topic, and a few existing studies have not reached a general conclusion about the ideal protocol. Some of the studies (19-22) have documented no significant differences between the different endometrial preparation protocols, and some studies (23-27) have indicated better pregnancy results for one protocol over the other.

According to Li et al. (19), HRT protocols had pregnancy outcomes similar to stimulated cycles (STC) for endometrial preparation. The available evidence shows that HRT may be a reasonable choice for the PCOS young women prepared for FET, who do not accept injections. On the contrary, STC may lead to reduced operational costs and unnecessary anxiety, and increased flexibility for patients. Najarkolaei et al. (20) reported no difference in abort rates and pregnancy outcomes between the mild-OS and the HRT protocols.

Peigne et al. (21) concluded that HRT and mild-OS groups showed comparable clinical pregnancy rates (20.8% vs. 24.4%). This study included women with PCOS i.e., about 20% of the patients.

The retrospective study by Yu et al. (22) showed similar endometrial thickness in the mildly stimulated cycle and HRT which resulted in non-statistically different rates of clinical and ongoing pregnancy and live birth. However, there was a significantly higher abortion rate in the mild-OS.

According to Man et al. (23), there is a significantly higher live birth rate in the PCOS women undergoing endometrial preparation during their initial FET cycle using the OS and NC methods using HRT. Nevertheless, there is a significantly higher rate of cycle cancelation in the NC group than in the other groups. the different groups do not show a significant difference in the rate of adverse events, such as preterm delivery, ectopic pregnancy, etc. This study has special significance since it is the first study on PCOS women.

Wang et al. (24) reported the better outcomes to HRT protocols. According to Niu et al. (25), both letrozole and Human Menopozal Gonadotropin (HMG) ovulation induction regimen had an association with more acceptable pregnancy results than the HRT regimen, such as a lower pregnancy loss rate, and a higher livebirth rate among the PCOS patients undergoing frozen single-blastocyst transfer.

Guan et al. (26) showed a higher live birth rate of the mild-OS and abort rates lower than the HRT in obese women with PCOS. This paper along with the available proof demonstrated superior pregnancy outcomes in the mild-OS than in the HRT. In a large retrospective study, Zhang et al. (27) reported significantly lower pregnancy loss rates of letrozole-stimulated cycles and higher live birth rates than the HRT protocol.

In this study, we acknowledge that we did not collect data about pregnancy difficulties, such as preeclampsia, gestational hypertension, and diabetes as the potential risk factors for adverse neonatal outcomes leading to a confounding effect on the results. The bias potential of medical records and the retrospective study design are the main limitations. The present study has the main strength which is the comparison of the most commonly used protocols for endometrial preparation in a large cohort of patients undergoing FET cycles, and the similarly increasing transferred good quality embryos in both HRT and mild-OS by the experienced clinicians and the verification method application in all embryos in a single center, affecting desirable outcomes of pregnancy.

CONCLUSION

This study revealed that pregnancy results, ongoing pregnancy rate and abort rate were similar among natural and artificial endometrial preparation protocols performed for FET cycles. Optimal endometrial preparation is necessary to receive successful pregnancy rates. Nevertheless, no statistical significance was found in the protocols. Dependent on clinical experience and facility, one of these protocols could be deployed for FET.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was carried out with the permission of Beykoz University Research and Project Development Ethics Committee (Date: 26.10.2020, Decision No:1).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

REFERENCES

1. Luo L, Chen M, Wen Y, Zhang L, Zhou C, Wang Q. Pregnancy outcome and cost-effectiveness comparisons of artificial cycle-prepared frozen embryo transfer with or without GnRH agonist pretreatment for polycystic ovary syndrome: a randomised controlled trial. BJOG 2021; 128: 667-74.
2. Thakre N, Homburg R. A review of IVF in PCOS patients at risk of ovarian hyperstimulation syndrome. Expert Rev Endocrinol Metab 2019; 14: 315-9.
3. Joham AE, Norman RJ, Stener-Victorin E, et al. Polycystic ovary syndrome. Lancet Diabetes Endocrinol 2022; 10: 668-80.
4. Dokuzeylül-Güngör N, Gürbüz T, Prediction of the number of oocytes based on AMH and FSH levels in IVF candidates J Surg Med 2020; 4: 733-7.
5. Güngör ND, Gürbüz T, Yurci A. Does depot analog suppression have positive effects on all other frozen-thawed embryo transfer cycles in addition to endometriosis. Ulutas Med J 2021; 7: 22-30.
6. Cerrillo M, Herrero L, Guillén A, Mayoral M, García-Velasco JA. Impact of endometrial preparation protocols for frozen embryo transfer on live birth rates. Rambam Maimonides Med J 2017; 8: e0020.
7. Mumusoglu S, Polat M, Ozbek IY, et al. Preparation of the endometrium for frozen embryo transfer: a systematic review. Front Endocrinol (Lausanne) 2021; 12: 688237.
8. Fatemi HM, Kyrou D, Bourgain C, Van den Abbeel E, Griesinger G, Devroey P. Cryopreserved-thawed human embryo transfer: spontaneous natural cycle is superior to human chorionic gonadotropin-induced natural cycle. Fertil Steril 2010; 94: 2054-8.
9. Groenewoud ER, Cantineau AE, Kollen BJ, Macklon NS, Cohlen BJ. What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? A systematic review and meta-analysis. Hum Reprod Update 2013; 19: 458-70.
10. Zhang Y, Wu L, Li TC, Wang CC, Zhang T, Chung JPW. Systematic review update and meta-analysis of randomized and non-randomized controlled trials of ovarian stimulation versus artificial cycle for endometrial preparation prior to frozen embryo transfer in women with polycystic ovary syndrome. Reprod Biol Endocrinol 2022; 20: 62.
11. Aghahoseini M, Alyasin A, Rashidi S, Samaei-Nouroozi A, Saeidi H, Shabani-Nashtaei M. The efficacy of gonadotropin-releasing hormone (GNRH) agonist before frozen embryo transfer in improving pregnancy outcome and decreasing miscarriage rate in hyperandrogenic polycystic ovary syndrome women: a randomized clinical trial. Minerva Ginecol 2020; 72: 212-8.
12. Mensing L, Dahlberg ES, Bay B, Gabrielsen A, Knudsen UB. Endometrial preparation methods prior to frozen embryo transfer: A retrospective cohort study comparing true natural cycle, modified natural cycle and artificial cycle. Arch Gynecol Obstet 2022; 306: 1381-8.
13. Demirdağ E, Güler İ, Cevher Akdulum MF, et al. Comparison of natural and artificial cycles in frozen-thawed embryo transfer: A retrospective analysis of 1696 cycles. Turk J Obstet Gynecol 2022; 19: 28-34.
14. Dokuzeylul Gungor N, Gurbuz T, Ture T. Prolonged luteal phase support with progesterone may increase papules and plaques of pregnancy frequency in pregnancies through in vitro fertilization. An Bras Dermatol 2021; 96: 171-5.
15. Dokuzeylül Güngör N, Güngör K, Yurci A, Cil K, Hatırnaz Ş. Ovarian drilling down-regulates endometrial nuclear factor-κB p65 expression in women with PCOS: A prospective case-control study. Turk J Obstet Gynecol 2022; 19: 45-50.
16. Giviziez CR, Sanchez EGM, Lima YAR, Approbato MS. Association of overweight and consistent anovulation among infertile women with regular menstrual cycle: a case-control study. Rev Bras Ginecol Obstet 2021; 43: 834-9.
17. Liu X, Shi J, Bai H, Wen W. Pretreatment with a GnRH agonist and hormone replacement treatment protocol could not improve live birth rate for PCOS women undergoing frozen-thawed embryo transfer cycles. BMC Pregnancy Childbirth 2021; 21: 835.
18. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*power 3.1: tests for correlation and regression analyses. Behav Res Methods 2009; 41: 1149-60.
19. Li L, Gao DD, Zhang Y, Song JY, Sun ZG. Comparison of stimulated cycles with low dose r-fsh versus hormone replacement cycles for endometrial preparation prior to frozen-thawed embryo transfer in young women with polycystic ovarian syndrome: a single-center retrospective cohort study from China. Drug Des Devel Ther 2021; 15: 2805-13.
20. Hosseini-Najarkolaei A, Moini A, Kashani L, Farid Mojtahedi M, Hosseini-Najarkolaee E, Salehi E. The effect of letrozole versus artificial hormonal endometrial preparation on pregnancy outcome after frozen-thawed embryos transfer cycles: a randomized clinical trial. Reprod Biol Endocrinol 2020; 18: 115.
21. Peigné M, Devouche E, Ferraretto X, et al. Higher live birth rate with stimulated rather than artificial cycle for frozen-thawed embryo transfer. Eur J Obstet Gynecol Reprod Biol 2019; 243: 144-9.
22. Yu J, Ma Y, Wu Z, et al. Endometrial preparation protocol of the frozen-thawed embryo transfer in patients with polycystic ovary syndrome. Arch Gynecol Obstet 2015; 291: 201-11.
23. Man Y, Bian Y, Zhao S, et al. The effect of different endometrial preparations on women with polycystic ovary syndrome undergoing initial frozen embryo transfer: A historical cohort analysis. Acta Obstet Gynecol Scand 2021; 100: 1116-23.
24. Wang Y, Hu WH, Wan Q, et al. Effect of artificial cycle with or without GnRH-a pretreatment on pregnancy and neonatal outcomes in women with PCOS after frozen embryo transfer: a propensity score matching study. Reprod Biol Endocrinol 2022; 20: 56.
25. Niu Y, Zhao D, Wang Y, Suo L, Zou J, Wei D. Ovulation induction regimens are associated with a higher rate of livebirth after frozen single-blastocyst transfer among women with polycystic ovary syndrome. Front Endocrinol (Lausanne) 2022; 13: 987813.
26. Guan L, Wu H, Wei C, et al. The effect of mildly stimulated cycle versus artificial cycle on pregnancy outcomes in overweight/obese women with PCOS prior to frozen embryo transfer: a retrospective cohort study. BMC Pregnancy Childbirth 2022; 22: 394.
27. Zhang J, Liu H, Wang Y, et al. Letrozole use during frozen embryo transfer cycles in women with polycystic ovary syndrome. Fertil Steril 2019; 112: 371-7.